Use of Rhu-GM-CSF in Pulmonary Tuberculosis Patients: Results of a Randomized Clinical Trial

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It has been postulated that deficient or incomplete clinical and/or microbiological response to tuberculosis treatment is associated with cell-mediated immunological dysfunction involving monocytes and macrophages. A phase 2 safety trial was conducted by treating patients with either recombinant human granulocyte-macrophage colony-stimulating factor (rhu-GM-CSF) or a placebo, both in combination with anti-tuberculosis chemotherapy. Thirty-one patients with documented pulmonary tuberculosis were treated with rifampin/isoniazid for six months, plus pyrazinamide for the first two months. At the beginning of treatment, rhu-GM-CSF (125μg/M^2) was randomly assigned to 16 patients and injected subcutaneously twice weekly for four weeks; the other 15 patients received a placebo. The patients were accompanied in the hospital for two weeks, then monthly on an out patient basis, for 12 months. Clinical outcomes were similar in both groups, with no difference in acid-fast bacilli (AFB) clearance in sputum at the end of the fourth week of treatment. Nevertheless, a trend to faster conversion to negative was observed in the rhu-GM-CSF group until the eighth week of treatment (p=0.07), after which all patients converted to AFB negative. Adverse events in the rhu-GM-CSF group were local skin inflammation and an increase in the leukocyte count after each injection, returning to normal 72 hours after rhu-GM-CSF injection. Three patients developed SGOP and SGPT > 2.5 times the normal values. All patients included in the GM-CSF group were culture negative at six months, except one who had primary TB resistance. None of the patients had to discontinue the treatment in either group. We conclude that rhu-GM-CSF adjuvant immunotherapy could be safely explored in a phase 3 trial with patients who have active tuberculosis.

Key Words: Tuberculosis, GM-CSF, treatment.

Tuberculosis (TB) remains an important health problem worldwide, with 8 million new cases annually, including 3.5 million cases of infectious pulmonary disease (smear positive), and 2 million deaths each year, despite considerable effort to control this disease. It is estimated that 1.9 billion people are infected worldwide, thus making TB one of the most prevalent infections in the world [1].

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The current treatment for TB in Brazil is short course chemotherapy with Isoniazid plus Rifampin for six months plus Pyrazinamide for the initial two months. Although the efficacy of this schedule is 95%, its efficiency is much lower, mainly due to noncompliance [2,3]. In Brazil, the default rate is 15% to 20% [4]. The default rate is even worse in countries that cannot afford the short course therapy [5].

Recombinant human granulocyte-macrophage colony-stimulating factor (rhu-GM-CSF) has been used to improve the immunological function of patients with various infectious diseases [6]. In a trial of rhu-GM-CSF treatment of neutropenic patients with visceral leishmaniasis (VL), it was found to be safe and efficient in reversing leukopenia associated VL, and it significantly reduced the number of secondary bacterial

infections [7]. The GM-CSF killing effect was demonstrated in an *in vitro* study of human monocyte-derived macrophages, as it inhibited the intracellular growth of a *Mycobacterium avium* complex strain [8]. Also, rhu-GM-CSF reduces the growth of *Mycobacterium* in human monocyte-derived macrophages [9].

We present the results of the first clinical trial that evaluated the safety of rhu-GM-CSF as adjuvant immunotherapy for the treatment of active human pulmonary tuberculosis.

Materials and Methods

Study population. Patients were included if they (1) presented with a clinical and radiological picture compatible with active pulmonary TB, and had strongly positive smears (3 plus), according to the acid-fast-bacilli (AFB) method in two consecutive sputum smears; (2) were between 18 and 50 years old; (3) had no history of previous TB treatment; and (4) had leukocyte counts below 25,000/mm^3 at entry. Patients were excluded if they (1) presented with any serious concomitant diseases, such as severe pulmonary dysfunction, renal, cardiac, hematological or hepatic diseases; (2) had severe dermatological lesions above grade 3 on the WHO score system; (3) were pregnant or lactating women; (4) had a history of alcohol abuse, had diabetes mellitus, mental disorders, were HIV or HTLV-I/II positive; (5) were receiving immunosuppressive treatments; or (6) could not provide informed consent or be followed after the hospitalization period. Administration of previous or concomitant medications such as antimycobacterial drugs, other than those initially scheduled, was not permitted during the study period.

All patients gave written informed consent prior to entering the study.

Randomization and study treatment. After diagnosis of tuberculosis, the patients who met all entrance criteria were hospitalized, for at least the first two weeks to allow better clinical evaluation of tolerability. Patients were randomized to take either rhu-GM-CSF 125 μg/M^2/dose) subcutaneously twice a week for four weeks, or a placebo, both supplied by Immunex Corporation. Neither the physician nor the patient were aware of which drug they were taking. In addition to the challenge treatment, all patients who weighed more than 45 kg were scheduled to take daily Rifampin (600mg/day) + Isoniazid (400mg/day) for six months, and Pyrazinamide (2000mg/day) for the initial two months. Patients below 45 kg were scheduled to take 450mg/day Rifampin + 300mg/day Isoniazid for six months, and 1500mg/day Pyrazinamide for the initial two months.

Assessment of patients and endpoints. Clinical evaluations included a medical history, a complete physical examination and laboratory tests. During the first two weeks, the patients were examined daily. At weeks three and four, they were examined on an outpatient basis, twice a week, and after week four, they were examined once a week until they became sputum-smear negative. Subsequent follow-up visits were made monthly up to the sixth month, and at 9 and 12 months after enrollment.

Sputum or induced sputum was collected on all visits to the clinic. Induction of sputum was performed with nebulization with 5ml of 20% saline solution for 20 minutes. Two laboratories independently received sputum samples for direct smear analysis and cultures. Direct smear analysis was done immediately after collection [10]. Cultures were made on standard Löwenstein Jensen medium with previous sputum centrifugation, decontamination, and neutralization, as described by Kubica-Dye [11]. The identification of *Mycobacterium tuberculosis* was done in accordance with technical guidelines. Sensitivity tests used the indirect proportion methods [12].

The primary endpoint was to evaluate the tolerance of patients with pulmonary TB to the GM-CSF immunotherapy. The secondary endpoint was the impact on the clearance of *M. tuberculosis* in the smear and culture.

Statistical analysis. It was estimated that 30 participants (15 per group) would be needed to provide

80% power and ≥0.95 probability to detect a 50% difference in relative difference in tolerability and number of side events between the two arms of treatment.

Ethical issues. The study was approved by the institutional review board of the University Hospital Professor Edgard Santos in Salvador, Bahia, Brazil, following the National Ethics regulatory guidelines of resolution 196/96 of the Brazilian Ministry of Health; it was conducted at the TB clinic of this hospital.

Results

Thirty-one patients were enrolled in the study. Patients in the two groups (rhu-GM-CSF and placebo) had comparable distributions of age, gender, weight, PPD induration, albumin, hemoglobin, BUN, SGOT, SGPT levels, and leukocyte counts at the start of the study (Table 1). Half of the rhu-GM-CSF group (8/16) and the placebo group (8/15) was male.

Overall, the 31 patients enrolled in the study were considered cured for active pulmonary TB at the end of the six-month treatment schedule. A stratified analysis of the parameters comparing the number of weeks of therapy revealedthat there were no significant differences between the two groups in the amount of time to negative AFB smears and TB cultures (i.e., absence of viable organisms capable of growing in culture media) (Figure 1). Nevertheless, a trend to faster conversion to negative was observed in the rhu-GM-CSF group until the eighth week of treatment ($p=0.07$), after which all patients had progressed to AFB negative. At week six, 7 of 14 patients treated with rhu-GM-CSF were culture negative, compared with 5 of 14 patients in the placebo group ($p = 0.44$). At week 12, all patients in both groups gave negative AFB and cultures (Figure 1).

Safety analysis. Table 2 presents data on the side effects during the first four weeks of rhu-GM-CSF or placebo use. The only significant difference observed was an increased frequency of cutaneous reactions, including pain and inflammation at the injection site in the rhu-GM-CSF group. Among the 16 patients who received GM-CSF, only one individual had two white blood cell counts within 24 hours of dose; both indicated leukocytosis (11,600 cells/mm^3 and 16,000 cells/mm^3). One patient died four months after starting the TB therapy. This patient did not have any side effects during the previous months, including the first month that she used rhu-GM-CSF. She did not have any pulmonary symptoms before death, and died suddenly after acute alcohol consumption. There was no significant difference in the frequency of adverse reactions between the two groups.

During the first eight weeks of therapy, the two groups had similar laboratory profiles of renal, hepatic, and hematologic parameters (Figures 2 and 3). Hepatic enzymes did not differ between the groups, and there was even a slight increase in mean SGPT in both groups during the second week of treatment. There was mild to moderate elevation of liver enzymes in six patients, randomly distributed in the placebo and GM-CSF groups. Hemoglobin and BUN tended to be stable during, and a slight increase in albumin levels in both groups was perceived (mean values from 3.3g/dl to 4.0g/dl in the rhu-GM-CSF group and from 3.4g/dl to 4.1g/dl in the placebo group).

Discussion

It was clearly shown that rhu-GM-CSF is safe and well-tolerated by patients with active TB. The clinical and laboratory outcomes were similar for patients using rhu-GM-CSF versus the placebo; the only diagnostic laboratory abnormality detected was increased liver enzymes, but it was randomly distributed in both groups, and was probably due to the drugs used for treating the TB [13, 14]. Pain and skin reaction were more frequent in those who received the GM-CSF injections, as expected [15]. Other side effects potentially associated with rhu-GM-CSF, such as fever and pulmonary necrosis, were not seen in this trial. Although patients were excluded if they had a history of alcohol abuse, one patient died after ingestion of a large amount of alcoholic beverage during 14 hours. In reviewing the episode with the family members, they indicated that she used to be an alcoholic, but had abstained

Table 1. Comparison of clinical, demographic and laboratory variables in tuberculosis patients treated with rhu-GM-CSF/triple therapy (GM-CSF group) and those treated with triple therapy alone (placebo group)

Variable	GM-CSF group X (SD)		Placebo group X (SD)		P-value[1]
Age (yrs)	31.6	(10.9)	28.0	(6.2)	ns
Weight (kg)	52.9	(10.9)	49.9	(7.8)	ns
PPD induration (mm)	19.4	(6.4)	19.6	(6.4)	ns
Duration of symptoms pre-treatment (weeks)	2.9	(2.6)	3.0	(3.0)	ns
WBC (total leukocytes)	8,425	(3,374)	10,693	(3,610)	ns
Hemoglobin (g/dl)	11.2	(1.2)	11.5	(1.8)	ns
Albumin (g/dl)	3.6	(0.4)	3.4	(0.4)	ns
AST (IU)	31.4	(28.2)	20.9	(6.1)	ns
ALT (IU)	34.2	(35.1	16.7	(7.2)	ns
BUN (g/dl)	25.7	(7.8)	22.9	(7.5)	ns

[1] T-test procedure for equal means; ns = Non-significant ($p>0.05$); WBC = White blood cell count; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; BUN = Blood urea nitrogen; SD = Standard deviation.

Table 2. Side effects observed during the first four weeks of rhu-GM-CSF or placebo subcutaneously in pulmonary tuberculosis patients treated with short-term therapy

Effect	Week 1		Week 2		Week 3		Week 4	
	GM	Placebo	GM	Placebo	GM	Placebo	GM	Placebo
Fever	0	0	1	1	1	0	1	0
Cutaneous reaction	4	1	6	1	5	1	0	1
Pain/inflammation, Vomiting	0	0	1	2	0	1	0	0
Neurological symptoms	0	0	0	1	0	0	0	0
Flu-like syndrome	0	1	0	0	0	0	1	0
Myalgia	0	0	0	0	1	0	0	0
Depression	0	0	0	1	0	1	0	1
Hemoptysis	0	0	0	0	0	1	1	0
Arthralgia	1	1	0	0	1	2	2	0

during the previous 24 months. The death occurred 10 weeks after the last dose of GM-CSF, and before death the patient was clinically stable, with no deviations in diagnostic laboratory results; she had a negative culture for *Mycobacterium tuberculosis*, so we do not think the death was related to the treatment.

Mycobacterium tuberculosis is an intracellular microbe for which the cell-mediated immune system plays an important role in the control of the infection.

The cytokines and T cell subsets implicated in the protection against *M. tuberculosis* and BCG are α-IFN, γ-IFN, and CD_8 lymphocytes [16]. The Th1 lymphocytes, cytotoxic T-cells, and NK cell-mediated cytotoxicity also play a role in the control of infections, as demonstrated by Kaufmann in murine macrophages infected with *M. tuberculosis* and *M. leprae* lysed by cytotoxic T-cells through an antigen-MHC process [16].

Figure 1. Decrease of positivity in bacterial cultures of patients treated with rhuGM-CSF or placebo plus short course chemotherapy

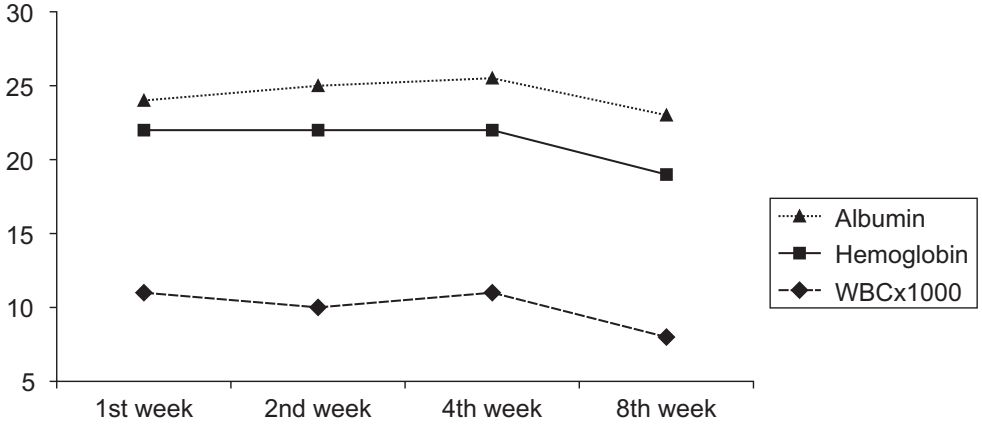


Figure 2. Laboratory profile during the first eight weeks in TB patients in the rhu-GM-CSF group

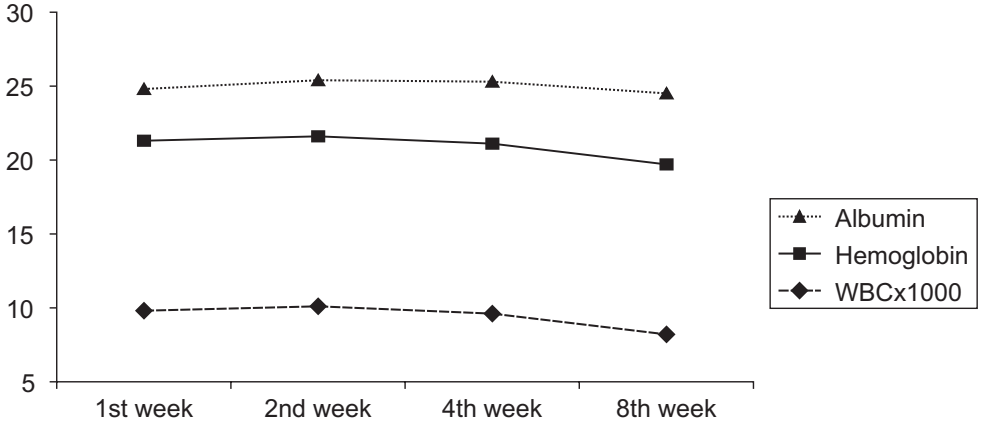
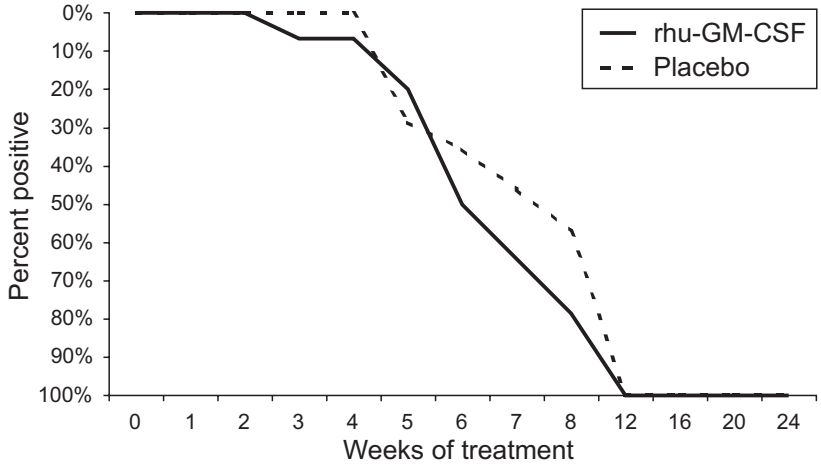


Figure 3. Laboratory profile during the first eight weeks in TB patients in the placebo group



Human granulocyte-macrophage colony-stimulating factor (hu-GM-CSF) is a cytokine, isolated by Nicola et al. [17,18]. It has been used against neoplastic diseases to promote the enhancement of macrophage tumoricidal activity, in the treatment or prophylaxis of leukopenia associated with myelo-suppressive therapies, and in patients with bone marrow failure [19,20]. Also, GM-CSF increases the number of leukocytes in the peripheral circulation and enhances granulocyte and monocyte function [21]. GM-CSF induces chemotactic activity by both polymorphonuclear and monocytic cells, and stimulates TNF-α synthesis and release [22]. The ability of GM-CSF to induce intracellular killing has been demonstrated against organisms such as *Leishmania species* [6,23,24], *T. cruzi* [25] and *Candida albicans* [26, 27]. TNF-mediated antimicrobial activity has been demonstrated in TB [8,9,28].

Bermudez et al. [28] examined the *in vitro* ability of rhu-GM-CSF to activate human monocyte-derived macrophages so that they inhibit the intracellular growth of, or kill, *Mycobacterium avium* complex (MAC) bacteria. They found that rhu-GM-CSF can activate macrophages to inhibit intracellular growth or kill MAC, and that killing can be augmented by TNF-α. More recently, Bermudez et al. [8] showed the effect of rhu-GM-CSF on disseminated MAC infection *in vivo* in a beige mouse model. A significant reduction in the number of viable bacteria was observed in the blood, livers, and spleens of mice treated with GM-CSF versus antimicrobials alone. Denis and Ghadirian [9] found that rhu-GM-CSF reduces the growth of *M. tuberculosis* in human monocyte-derived macrophages *in vitro*, as compared to untreated cells. The decrease in intracellular growth of the tuberculous bacilli did not depend on the generation of reactive oxygen species.

The placebo and GM-CSF groups had the same clinical and laboratory outcomes. The elevation of liver enzymes was not associated with the use of rhu-GM-CSF. This toxicity was probably provoked by the TB therapy [13]. Hemoglobin, BUN, and WBC tended to be stable, and a slight increase in albumin levels in both groups was probably due, in part, to better nutrition during hospitalization. Also, fever, weight loss, increased pulmonary necrosis, and increased pulmonary fibrosis associated with GM-CSF therapy that had been documented elsewhere [15,29], were not seen in the rhu-GM-CSF treatment group.

No differences in treatment efficacy were observed between the two groups. The time to negative results in smears and cultures was similar for the two groups. If there was a difference in the efficiency of eliminating mycobacteria with the rhu-GM-CSF regimens, it was not seen with this sample size, and another study design is necessary. However, in one patient in the rhu-GM-CSF group, the *M. tuberculosis* isolated in the sputum at the beginning of treatment was multiresistant to the triple drug treatment. This patient promptly responded to the therapy, similar to all the others with sensitive *M. tuberculosis* isolates. It is possible that the predicted enhanced killing effect of GM-CSF is responsible for the successful treatment of this particular patient, even though he was infected with a drug-resistant microorganism. On the other hand, the lack of differences in the rate of conversion to negative in the clearance of AFB in both groups might be explained by the fact that the efficiency of DOT (directly observed) therapy for patients with sensitive organisms is usually greater than 90% [30,31]. Patients with disseminated TB, including those with HIV coinfection, might benefit from treatment with adjuvant GM-CSF therapy. It is well known that the incidence of resistant TB organisms in this patient population is increasing [32], although this seems to be correlated with other factors [33].

The safety profile of this preliminary trial with the combination of anti-TB therapy with rhu-GM-CSF offers an opportunity to explore the benefit of this adjuvant therapy for the treatment of TB caused by resistant microorganisms in phase 3 trials.

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