Thalidomide Treatment Reduces Tumor Necrosis Factor α Production and Enhances Weight Gain in Patients with Pulmonary Tuberculosis

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ABSTRACT

Background: The monocyte-derived cytokine, tumor necrosis factor α (TNFα), is essential for host immunity, but overproduction of this cytokine may have serious pathologic consequences. Excess TNFα produced in pulmonary tuberculosis may cause fevers, weakness, night sweats, necrosis, and progressive weight loss. Thalidomide (α-*N*-phthalimidoglutarimide) has recently been shown to suppress TNFα production by human monocytes in vitro and to reduce serum TNFα in leprosy patients. We have therefore conducted a two-part placebo-controlled pilot study of thalidomide in patients with active tuberculosis to determine its effects on clinical response, immune reactivity, TNFα levels, and weight.
Materials and Methods: 30 male patients with active tuberculosis, either human immunodeficiency virus type 1 positive (HIV-1^+) or HIV-1^-, received thalidomide or placebo for single or multiple 14 day cycles. Toxicity of the study drug, delayed type hypersensitivity (DTH), cytokine production, and weight gain were evaluated.
Results: Thalidomide treatment was well tolerated, without serious adverse events. The drug did not adversely affect the DTH response to purified protein derivative (PPD), total leukocyte, or differential cell counts. TNFα production was significantly reduced during thalidomide treatment while interferon-γ (IFNγ) production was enhanced. Daily administration of thalidomide resulted in a significant enhancement of weight gain.
Conclusions: The results indicate that thalidomide is well tolerated by patients receiving anti-tuberculosis therapy. Thalidomide treatment reduces TNFα production both in vivo and in vitro and is associated with an accelerated weight gain during the study period.

INTRODUCTION

Tumor necrosis factor α (TNFα) is one of the important immunologic mediators generated by cells of the monocyte/macrophage lineage and has been shown to have significant effects on host immunity in bacterial and parasitic infections (1). Tuberculosis is a chronic mycobacterial infection (of macrophages and monocytes) in which TNFα is characteristically produced. TNFα is present in the pleural effusions of patients infected with *Mycobacterium tuberculosis*, and monocytes isolated from patients with active dis-

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ease and systemic symptoms release elevated amounts of TNFα into the culture supernatant in response to stimuli (2–4). In vitro, mycobacterial preparations have been shown to induce TNFα production by human monocytes (5). TNFα is essential for granuloma formation (6,7), which is intimately associated with resistance against mycobacteria (8).

While production of TNFα is essential for host immunity, overproduction of this cytokine may have serious pathologic consequences. In experimental animals, high levels of TNFα induce fever by direct action on the hypothalamus (1), and long-term administration of this cytokine causes marked losses of fat and muscle cells or cachexia (9). Elevated levels of TNFα produced in pulmonary tuberculosis may be responsible for many of the pathologic symptoms of the disease, including fevers, weakness, fatigue, night sweats, lung necrosis, and progressive weight loss (10).

The control of these pathologic effects of excess TNFα production has received increasing attention. In animal models of gram-negative sepsis, administration of either antibodies directed against TNFα or pharmaceutical agents that suppress TNFα production, leads to reduced fevers, less wasting, and significantly decreased mortality (11). One drug, thalidomide (α-*N*-phthalimidoglutarimide), has recently been shown to suppress TNFα production by human monocytes in vitro (12,13) and to normalize elevated levels of serum TNFα in vivo (12,14). The inhibitory activity of thalidomide is due to a selective destabilization of the TNFα mRNA (15); thalidomide selectively suppresses TNFα production in vitro without directly affecting the levels of other cytokines such as interleukin-1 (IL-1), IL-6, and granulocyte/macrophage-colony stimulating factor (GM-CSF) (13).

Since thalidomide inhibits but does not abolish TNFα production, thalidomide therapy might reduce the toxicities linked with overproduction of the cytokine, without interfering with its important role(s) in host immunity. Recent studies have shown that thalidomide has prompt and dramatic effects when given to leprosy patients with erythema nodosum leprosum (ENL), including the elimination of the lesions, a decrease in inflammation, and general enhancement of patient well-being. These studies suggest that thalidomide may play a therapeutic role in tuberculosis patients, where much of the pathology may be TNFα mediated, but where a normal immune response and granuloma formation is important in the control of the infection.

In order to determine the effect of thalidomide on TNFα levels and whether thalidomide treatment is associated with any untoward side effects, including drug toxicity or suppression of immune reactivity, we have conducted a two-part placebo-controlled pilot study of thalidomide in patients with active tuberculosis. The results of our study indicate that thalidomide reduces TNFα production by peripheral blood mononuclear cells (PBMCs) in patients receiving anti-tuberculosis therapy without any adverse effects. Furthermore, although all patients showed the normal expected response to anti-tuberculosis therapy, only patients treated with thalidomide demonstrated significant increased weight gain during the study period.

MATERIALS AND METHODS

Study Drug

Thalidomide (CG-217) was provided in 100-mg tablets (Lot 204 and 206) by Grunenthal GMBH (Aachen, Germany). Placebo tablets were also provided by Grunenthal GMBH (Aachen, Germany).

Study Design

A total of 30 male patients with active tuberculosis, either HIV-1^+ or HIV-1^-, were studied. Twenty of the patients were studied in Chiang Mai, Thailand (Group I), and 10 were studied in New York City, U.S.A. (Group II). In Thailand, patients were evaluated at the Tuberculosis Center in Chiang Mai and admitted to the Tuberculosis Sanitorium. In New York, patients were transferred from Bellevue Hospital to the Clinical Research Center (CRC) at the Rockefeller University Hospital.

All patients received 300 mg of thalidomide or placebo in tablet form (3 tablets) daily at bedtime. The Thailand patients received only a single 14-day cycle of thalidomide or placebo. In New York, after the first treatment cycle with thalidomide or placebo, Patients 25–30 were allowed a 7-day washout period and then treated with a second, third, and in some a fourth cycle of thalidomide or placebo. Of the 10 New York patients, one completed only 7 days of thalidomide during the initial cycle of treatment (Patient 21); the remaining nine completed the full initial cycle of 14 days of thalidomide or placebo.

INCLUSION CRITERIA. Table 1 summarizes the patient characteristics. Patients were included in the study if they had acid fast organisms on sputum examination with eventual growth of *M. tuberculosis* in culture and met one or more of the following criteria: fever greater than 38°C; weight loss greater than 5 kg; night sweats; and evidence of pulmonary disease on chest X-ray consistent with tuberculosis. Patients were excluded from the study if there was growth of mycobacteria other than tuberculosis or if patients refused human immunodeficiency virus type 1 (HIV-1) testing. Also patients with pre-existing peripheral neuropathy were excluded, since this is a well-described side effect of thalidomide.

CONCOMITANT THERAPIES. All patients received multidrug anti-tuberculosis regimens (MDT) as shown in Table 1. Anti-retroviral therapy was administered to the New York patients who were HIV-1^+ (Table 1) but was not available for the Thailand patients. The protocol was approved by the Institutional Review Board of the Rockefeller University Hospital and by the Ministry of Health in Thailand. Written consent was obtained for all patients.

Evaluation of Patients

All patients were evaluated by history and physical exam at baseline, at multiple time points during the study, and after cessation of the drug. Vital signs, including weight, were obtained twice daily. In Thailand, complete blood counts with differentials and biochemistry profiles were obtained before, on Day 7 of treatment and 5 days after the end of drug treatment. In New York, in addition to complete blood counts and biochemistry profile, laboratory evaluation included phenotyping of blood mononuclear leukocytes. Chest radiographs were obtained at baseline and after cessation of treatment with thalidomide or placebo. Delayed type hypersensitivity (DTH) was assessed by the response to intradermal injection of 0.1 ml of the tuberculin PPD (Mantoux, Connaught Laboratories, Inc., Swiftwater, PA, U.S.A.) and quantitated after 48 hr. DTH testing was performed at baseline, on Day 7 of treatment with drug or placebo, and 5 days after discontinuation of thalidomide or placebo. The size of the response is expressed as the mean of the two greatest diameters (in mm). Anergy testing was performed using the Multitest skin test reaction (Connaught). Patients 20, 23, 25, and 26 were not available for repeated skin testing. Complete caloric intake was recorded for patients in New York (Group II) only.

PBMC PHENOTYPING. PBMCs were isolated as previously described (15). For indirect immunofluorescent staining, PBMCs were stained with a saturating amount of the following monoclonal antibodies: anti-CD3 (Leu-4), anti-CD4 (Leu-3a), anti-CD8 (Leu-2a), anti-CD19 (Leu-12) (Becton Dickinson, Mountain View, CA, U.S.A.), anti-CD56 (NKHI) (Coulter, Hialeah, FL, U.S.A.), and anti-CD14 (Ortho Diagnostics, Westwood, MA, U.S.A.). Cells were then analyzed with the Becton Dickinson FACScan flow cytometer.

Tissue Culture

PBMCs were isolated from blood obtained from all patients in the study and resuspended at a density of 2×10^6/ml in 2× culture medium (CM). CM consisted of RPMI 1640 (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% pooled human AB^+ serum (Biocell, Carson, CA, U.S.A.), 100 U penicillin/ml, 100 μg streptomycin/ml, and 2 mM L-glutamine (Gibco). Incomplete tissue culture results were obtained for Patients 21–23 because of tissue culture contamination; not all assays were available for Patients 20 and 30.

Cytokine Measurements

IN VITRO. Bacterial preparations: The Pasteur strain 1011 of *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) (Ataufo de Paiva Foundation, Rio de Janeiro, Brazil), the purified protein derivative (PPD) of *M. tuberculosis* for use in vitro (Statenseruminstitut, Copenhagen, Denmark), synthetic muramyl dipeptide (MDP) (Sigma, St. Louis, MO, U.S.A.), and gram-negative lipopolysaccharide isolated from *Salmonella typhimurium* (LPS) (List Biological Laboratories, Campbell, CA, U.S.A.) were obtained commercially.

For induction of cytokines, 0.1-ml aliquots of cell suspensions were added to each well of 96-well flat bottom tissue culture plates (Costar, Cambridge, MA, U.S.A.) containing 0.1-ml aliquots of RPMI 1640 alone, or bacterial preparations suspended in RPMI 1640, to give final concentrations of 10 μg PPD, 10 μg BCG, 0.5 μg MDP, or 10 ng LPS per ml.

Plates were incubated for 16 hr at 37°C, in 5% CO_2. Then enzyme-linked immunosorbent assays (ELISA) (Endogen, Boston, MA, U.S.A.), were performed according to the manufacturer's

TABLE 1. Patient characteristics, clinical status, and therapies

Patient Number	Study Drug[a]	HIV-1 Status	Age (years)	Baseline Weight (kg)	No. Lung Fields Involved[b]	Medication[c]
Thailand						
1	t	+	43	48.0	6	Rifater, E
2	t	+	51	49.0	4	Rifater, E
3	t	−	32	43.0	4	Rifater, E
4	t	−	65	40.5	4	Rifater, E
5	t	−	46	43.3	4	Rifater, E
6	t	+	60	41.3	4	RPE
7	t	−	31	52.8	1	RIP
8	t	+	38	48.3	2	Rifater, E
9	t	−	25	56.5	3	Rifater, E
10	t	+	25	49.5	1	Rifater, E
11	t	+	49	44.5	3	Rifater, E
12	t	−	38	50.5	2	Rifater, E
13	p	−	25	40.0	2	Rifater, E
14	p	−	53	50.0	4	Rifater, E
15	t	−	55	52.0	5	Rifater, E
16	p	−	64	49.0	5	Rifater, E
17	t	−	22	42.0	1	Rifater, E
18	p	−	46	63.3	1	Rifater, E
19	t	−	63	71.0	3	Rifater, E
20	p	−	42	58.0	NA	Rifater, E
New York						
21	t	+	44	63.3	1	RIPE + Anti retroviral
22	t	+	37	71.0	2	RIPE + Anti retroviral
23	p	+	33	77.7	1	RIPE + Anti retroviral
24	p	+	45	76.5	3	RIPE + Anti retroviral
25	t;t;p;t	−	32	53.7	3	RIPE
26	t;t	+	35	66.5	2	RIPE + Anti retroviral
27	t;t	+	30	63.3	3	RIPE + Anti retroviral
28	t;t;p;t	−	38	74.5	3	RIPE + CIP¶ + ETH¶
29	p;t;t	+	42	83.8	1	RIPE + Anti retroviral
30	t;p;t;p	−	46	89.6	2	RIPE

NA, not available; R, Rifampin; I, Isoniazid; P, Pyrazinamide; E, Ethambutol; CIP¶, Cipro for 16 days; ETH¶, Ethionamide for 16 days.

[a]Patients received either thalidomide (t), placebo (p), or multiple cycles of t or p in the sequence shown.

[b]Infiltration, granulomas, or cavities present on chest X-ray: a total of six lung fields were evaluated and results given for each patient.

[c]Rifater, Rifampin isoniazid pyrazinamide.

instructions on 0.1-ml aliquots of culture supernatants, or of 10-fold dilutions of the same supernatants, to determine TNFα, IL-1, and interferon-γ (IFNγ) levels. All data points were derived from triplicate cultures.

DIRECT ASSAY OF CYTOKINES IN PATIENT SERA. Samples of serum obtained from the patients at the same time points as PBMCs were assayed directly for TNFα, IL-1, and IFNγ by ELISA (Endogen). All assays were performed in triplicate. Serum samples were also assayed for total TNFα (receptor bound as well as free bioactive TNFα) by a commercial EIA (Medgenix, Fleurus, Belgium) (16).

Lymphocyte Transformation Tests

Culture conditions for lymphocyte transformation tests (LTTs) were identical to those described above except that U-bottom plates were used. After 5 days of incubation, 1 μCi of tritiated thymidine ([^{3}H])-TdR) (New England Nuclear, Boston, MA, U.S.A.) was added to U-bottom microwells. Following a 16-hr incubation, wells were harvested onto paper mats which were thoroughly washed, dried, and immersed in scintillation fluid for counting β emission. Data is also presented as stimulation index (SI) calculated as: (the cpm in the presence of stimulus)/(cpm in the CM alone).

Quantitative Reverse Transcriptase-Polymerase Chain Reaction Analysis of Cytokine mRNA Levels

Freshly isolated PBMCs obtained from all group II patients (New York) were used. RNA isolated from PBMCs obtained from Group I patients (Thailand) was partially degraded and not used for analyses. Reverse transcriptase-polymerase chain reaction (RT-PCR) for cytokine mRNA was carried out as described in detail elsewhere (17). Briefly, RNA was extracted from 4–6 × 10^6 PBMC immediately after isolation using RNAzol B (Cinna/Biotecx, Houston, TX, U.S.A.) according to the manufacturer's instructions. Absorption spectroscopy was used to measure purity and concentration of RNA with a $A_{260/280}$ ratio of 2.0 indicating highly purified RNA. RNA was reverse transcribed into cDNA which was then amplified using the Gene-Amp RNA-PCR kit (Perkin Elmer Cetus, Norwalk, CT, U.S.A.) with 30 cycles of 95°C for 1 min (denaturation) and 60°C for 1 min (primer annealing and extension). To normalize for the amount of input RNA, RT-PCR was performed on the constitutively expressed gene encoding glyceraldehyde phosphate dehydrogenase (GA3PD). PCR products were electrophoresed and transferred to nylon membranes (Zetabind, Cuno, Inc., Meriden, CT, U.S.A.). After prehybridization, membranes were hybridized with 5 × 10^5 cpm/ml of ^{32}P-labeled oligonucleotide probes for 8–12 hr at 40°C. After washing, membranes were exposed to X-ray film (Hyperfilm-MP, Amersham Corp., Arlington Heights, IL) at room temperature with an intensifier screen. Quantitative analysis by comparison with known standards, and primer and probe sequences have been detailed (17).

Statistical Analysis

Data obtained were evaluated by the Wilcoxon signed rank test (two-sided test), or by the Student's paired sample *t* test. *p* values of 0.05 or less were considered significant.

RESULTS

Drug Tolerance and Adverse Effects

At the inception of the study, 10 male patients (Table 1) were enrolled in Group I (Thailand). The patients had been recently diagnosed with active tuberculosis and all began anti-tuberculosis treatment with MDT simultaneously or within a few days of treatment with thalidomide. All patients received one 14-day cycle of thalidomide, as described in Materials and Methods. The thalidomide treatment was well tolerated. No peripheral neuropathy, excessive sedation, or other adverse events were observed. The only side effects noted were morning drowsiness, dry mouth, and constipation. In addition, all patients responded well to concomitant anti-tuberculosis therapy and showed clinical improvement.

Since there were no adverse effects, approval was granted for enrollment of an additional 10 patients in the study (in Thailand). These 10 additional male patients (Table 1) were randomized to receive either thalidomide or placebo. All patients completed the 14-day cycle of thalidomide or placebo treatment. Administration of either thalidomide or placebo did not result in any clinical or radiographic deterioration in any of the patients. Again, clinical improvement was noted after beginning anti-tuberculosis therapy. One patient (Patient 6, Table 1) developed a macular rash which resolved with discontinua-

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